THE OCCURRENCE OF BACTERIA IN FROZEN SOIL

E. C. Harder

(with two graphs)

Introduction

The results of various investigators, notably Conn and Brown, have shown that the actual number of bacteria in the soil often increases with a decrease in temperature. This phenomenon is not confined to a gain in the number of bacteria alone, but is accompanied by a stimulation of the activity of the microorganisms. Many bacteriological processes, as ammonification, denitrification, and free nitrogen fixation, have shown an increase in frozen soils.

Because of the practical importance of this problem for agricultural practice it was thought advisable to note the effect of cold and moisture on the number of bacteria in Madison soil. Here the variations in temperature are greater than those recorded by former investigators.

In order to study what effect these great variations would exert on the number of bacteria, a series of plate counts was made. If the number of bacteria in the soil increases with freezing, there should be a parallel increase in available plant food. Many questions arise. Do all soils when sampled in the winter show the same general increase? What effect does stimulation of bacteria have on soil fertility? It has been noted that an increase in bacterial activity results in an increase in food for higher plants. The increase in the number of bacteria during the winter probably plays an important part in soil fertility. This is decidedly at variance with the earlier idea that frozen soils are dormant.

Previous work

The more recent experiments on the activity of bacteria in frozen soil were conducted by Conn[1] and Brown and Smith.[2]

[1] Conn, H. J., Bacteria in frozen soil. I and II. Centralbl. Bakt. **28**:422–434. 1910; **32**:70–97. 1911.

———, Bacteria of frozen soil. N.Y. Agric. Exp. Sta. Tech. Bull. 35. July 1914.

[2] Brown, P. E., and Smith, R. E., Bacterial activities in frozen soils. Agric. Exp. Sta. Iowa State College of Agric. Research Bull. 4. January 1912.

CONN's experiments dealt mainly with the number of bacteria in frozen soils, which he compared with the number in the same soils when in an unfrozen condition. He worked both with field and with potted soils and reached the following conclusions: (1) "the increase in number of bacteria after freezing is not due to the increase in soil moisture which usually occurs in winter"; (2) "the same increase in germ content may take place in potted soil, where there is no possibility that the bacteria are carried up mechanically from lower depths during the process of freezing"; and (3) this phenomenon is probably "due to an actual growth of bacteria after the soil is frozen."

The experiments were performed with the samples of field soil taken from plates prepared for the purpose, both aerated and unaerated soils being examined. Each type of soil, therefore, probably had a nearly uniform bacterial content originally throughout its mass, and the changes that occurred in it from time to time must be ascribed to the effect upon it of changes in the atmosphere. Unfortunately, samples were taken only at irregular intervals, so that, while the results show a general higher content of bacteria in frozen soils during the winter, they are not sufficiently detailed to show definitely when such increases occurred and to what they were due.

It is suggested that possibly freezing may have the effect of breaking up compact colonies which under ordinary conditions would not separate into individuals, thus making the increase only an apparent one, owing to more individuals producing separate colonies on the plates. This view is discarded, however, because upon the thawing of the soil the number of bacteria again decreases to practically what it was before freezing. It is claimed also that the maximum number is reached several weeks after a frost.

CONN concluded, therefore, that the increase is due to actual multiplication, and the supposition is that such multiplication takes place in certain denser portions of the soil solution, which, as suggested by BROWN and SMITH (*loc. cit.*), probably do not freeze. It is suggested that conditions in these unfrozen portions may favor the growth of certain prolific kinds of bacteria and suppress the growth of other varieties which at ordinary temperatures interfere with the growth of these prolific kinds.

An analogous hypothesis proposed by RUSSELL[3] for increases in the number of bacteria after partial sterilization by heat, frost, or other means is that by such partial sterilization the protozoa are killed, thus permitting the unhindered development of bacteria which under ordinary conditions is held in check by protozoa.

BROWN and SMITH (*loc. cit.*) in their investigations dealt mainly with the physiological activities of bacteria under conditions of low temperature and frost, although they also made some determinations of the number of bacteria in frozen soil. Their principal conclusions regarding the ammonifying, nitrifying, denitrifying, and nitrogen fixing powers of frozen soils are as follows: (1) that "frozen soils possess a much greater ammonifying power than unfrozen soils"; (2) that "during the fall season, the ammonifying power of the soil increases until the temperature of the soil almost reaches zero, when a decrease occurs, and this is followed by a gradual increase and the ammonifying power of the soil reaches a maximum at the end of the frozen period"; (3) that "the nitrifying power of frozen soils is weak and shows no tendency to increase with extension of the frozen period"; (4) that "frozen soils possess a decided denitrifying power which seems to diminish with the continuance of the frozen period"; (5) that "during the fall season, the denitrifying power of the soil increases until the soil freezes, after which a decrease occurs"; (6) that "frozen soils possess a nitrogen fixing power which increases with the continuance of the frozen period, being independent of moderate changes in the moisture conditions, but restricted by large decreases in moisture"; and (7) that "in the fall, the nitrogen fixing power of the soil increases until the soil becomes frozen, when it almost ceases, after which a smaller nitrogen fixing power is established."

The experiments on which these conclusions are based were conducted with air-dried soil of uniform texture and composition as a medium. This soil was enriched with suitable materials for ammonification, nitrification, denitrification, or nitrogen fixation.

[3] RUSSELL, E. J., The effect of partial sterilization of soil on the production of plant food. Jour. Agric. Sci. 5:152–221. 1913.

One-hundred gm. samples of soil thus prepared were then inoculated with infusions consisting of a mixture of the fresh soils to be tested and sterile water, 10 cc. of the infusion (containing in each case about 5 gm. soil) being added to each 100 gm. sample. The final moisture content of the soils during incubation was 20 per cent.

BROWN and SMITH assert that actual multiplication in the numbers of bacteria takes place in frozen soils, and that to this must be ascribed the increased numbers during the winter months. They present the hypothesis that, owing to the concentration of various salts in the film of hygroscopic water around soil particles, this film probably does not freeze under ordinary winter conditions, and bacteria may live and multiply in it.

Present investigations

SUMMARY OF RESULTS.—During the winter of 1914–1915, the writer conducted a series of experiments with field and potted soils. The results obtained justify conclusions which are somewhat at variance with those obtained during the recent investigations by CONN at Ithaca and Geneva, New York, and by BROWN and SMITH at Ames, Iowa; and it seems advisable, therefore, to publish them. The principal results obtained were as follows:

1. It was found that the number of bacteria in surface soil increased markedly after heavy frosts and in general maintained a high average during the winter months. The increases and decreases, however, were found to bear a distinct relation to the moisture content.

2. The potted soils failed to show such marked increase in bacterial content after frosts. On the contrary, the enriched cultures showed a distinct retardation of bacterial growth when in a frozen condition.

3. The bacterial flora was more or less the same during the fall, winter, and spring, with the exception that after heavy frosts the small transparent colonies characteristic of water and of deeper soils formed a larger proportion of the growth on the plates.

From these results it seems reasonable to conclude that ordinary soil bacteria undoubtedly withstand cold to a marked degree, even to temperatures as low as 4° C. or more below zero. The increase in numbers, however, seems to be due to mechanical transportation by moisture coming up from below during heavy frost, and where such transportation is not possible there is an actual retardation in growth as compared with that in unfrozen soils.

EXPERIMENTS WITH FIELD SOIL.—The investigations were begun during the latter part of October, and the samples were examined every week until the latter part of February, after which time the samples were examined at greater intervals.

A dark, medium rich, slightly sandy garden soil was studied. It was obtained from the university campus near the College of Agriculture, and care was taken to secure successive samples from exactly the same place. The soil was sampled to a depth of about 6 inches each time, thus giving an average of a surface layer to this depth. The temperature of the soil was taken roughly with an ordinary thermometer.

The entire sample was thawed, when necessary, and thoroughly mixed in a mortar, previously washed out with 95 per cent of alcohol. Twenty gms. of soil were then mixed with 400 cc. of sterile water, and from this 25 cc. were carried to a second 400 cc. sterile water blank, and so on to the fourth dilution. From the fourth or fifth dilution plates were poured and these were counted after an incubation period of 8 days at 28° C. Heyden Nährstoff agar was used as a culture medium.

It appears that in order to obtain conclusive results regarding the bacterial content of soils, samples should be investigated at short intervals of time, perhaps every few days, or even every day, as at times of heavy frost, or after rainfall. It was found that the bacterial content of soils is closely dependent on atmospheric conditions as regards temperature and precipitation. When atmospheric conditions vary rapidly the bacterial content of soils also may change rapidly. Table I shows the variations in the bacterial content of the soil from October 1914 to April 1915.

Table I shows the general close relation between the moisture content and the number of bacteria in the soil. This is also shown in graph 1. While slight discrepancies undoubtedly occur, the general correspondence is very marked. Graph 2 gives the curves of high and low daily temperature, as well as figures for the precipitation during the period that the experiments were carried on.

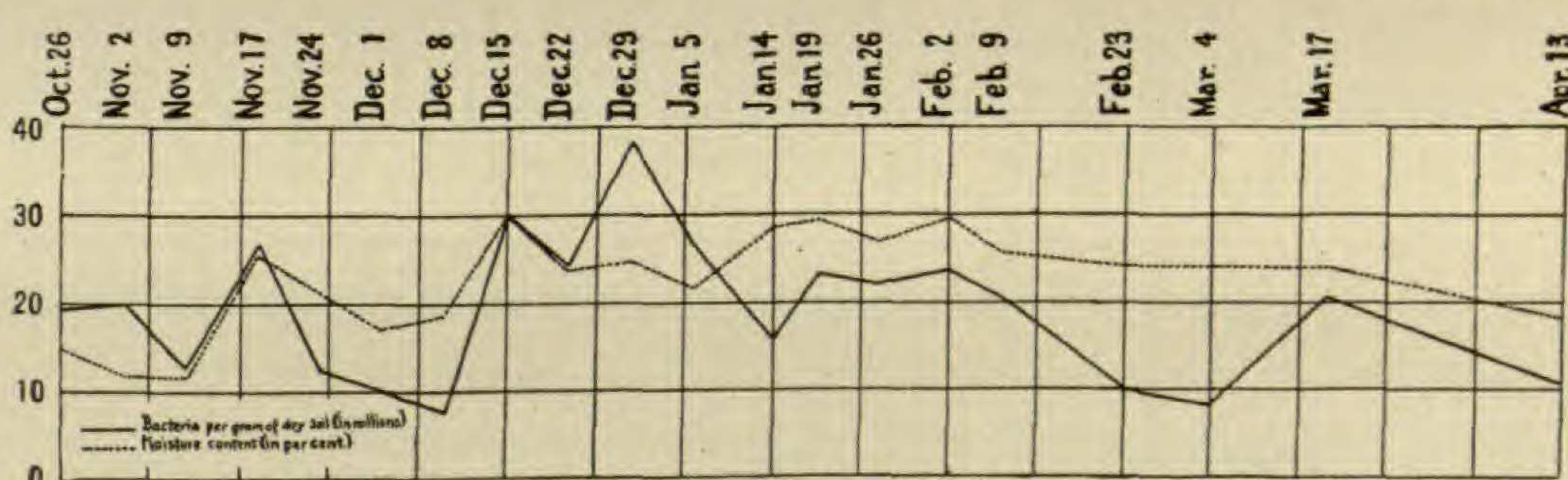
It will be seen by an examination of table I that there was some variation in the bacterial content during the latter part of October

TABLE I

Date	Percentage of moisture content of soil	Tempera-ture of soil	Average number of colonies per plate*	Bacteria per gm. dry soil	Remarks
1914					
October 26..	15.02	....	168	19,420,800	Soil unfrozen
November 2..	11.83	....	182	20,187,358	Soil unfrozen
9..	11.79	....	114	12,692,786	Soil unfrozen
17..	25.64	−0°.5C.	203	26,824,623	Soil frozen 4–5 inches
24..	20.98	0.0	100	12,434,800	Upper 1 inch thawed; below frozen
December 1..	17.02	+8.0	81	9,534,105	Soil completely thawed
8..	18.44	+1.0	62	7,469,450	Rain December 7
15..	30.05	−3.0	212	29,781,548	Frost December 9 Heavy frost December 13
22..	23.48	−4.0	188	24,018,316	Snowfall December 20; continued frost
29..	24.48	−0.7	293	38,122,523	Heavy frost December 25–26; heavy snowfall December 29
1915					
January 5..	21.52	−1.0	213	26,668,239	Mild temperature
14..	28.62	−1.0	115	15,830,555	Mild temperature
19..	29.24	−3.0	166	23,051,258	Thaw and subsequent frost
26..	26.87	−2.7	164	22,035,532	Cold weather January 21–24
February 3..	29.53	−1.0	168	23,425,080	Very severe frost January 27–28
9..	25.60	−3.0	153	20,206,710	Thaw and rain February 4–5
23..	24.03	+1.0	76	9,824,216	Soil completely thawed
March 4..	24.10	−0.7	65	7,993,570	Upper 3 inches frozen
17..	24.00	+0.5	157	20,298,373	Upper ½ inch frozen
April 13..	18.40	+4.0	89	10,717,113	Completely thawed

* Fourth dilution

and the first part of November, while the soil was still unfrozen. This variation seems to have been independent of the moisture content. On November 17, some days after the first frost, a distinct increase in the number of bacteria was shown, accompanied by a great increase in moisture. During the subsequent period of mild weather continuing to December 9, the bacterial content of the soil gradually decreased to much below the original amount, the lowest count of the winter, 7,459,450 bacteria per gm. of dry soil, occurring during this period. At the same time the soil thawed and the moisture content decreased. The count of December 15, taken some days after the second frost, again showed a marked increase in bacterial numbers, as well as in moisture content. Following this there was a continuous cold period culminating in very heavy frost from December 25 to 27. On December 29, following this heavy frost, we had the highest bacterial count of the winter, 38,122,523 bacteria per gm. of dry soil. The next count, January 5, showed a considerable decrease with but a slight decrease in moisture content. This is probably to be explained

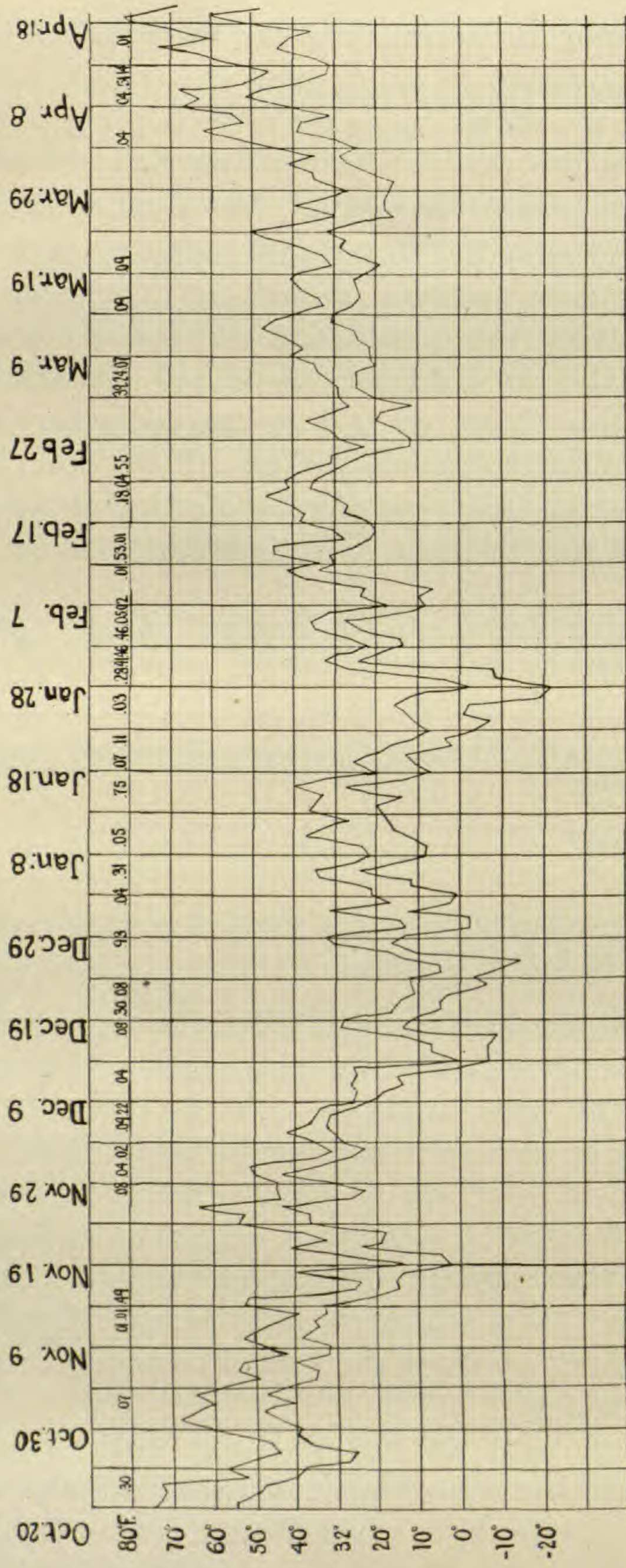


GRAPH 1.—Curves showing the bacterial and moisture contents of Madison soil from October 26, 1914, to April 13, 1915.

by the fact that the high count of December 29 included many forms that could not withstand the more severe cold to which they were exposed in the upper soil layer. The low count of January 14 accompanied by an increase in moisture is difficult to explain.

After this date there was no great variation in the bacterial content until February 23, when there occurred a marked decrease accompanying the complete thawing of the soil. During this thawing, however, there was only a slight decrease in soil moisture. The figure for April 13 shows the normal bacterial content of the soil during the spring.

EXPERIMENTS WITH POTTED SOIL.—In order to determine whether the high bacterial content of frozen soil was due to an actual increase in growth, or whether other factors brought about this phenomenon, two duplicate sets of potted soil were prepared.



GRAPH 2.—Curves showing high and low daily temperatures at Madison, Wisconsin, and figures giving precipitation from October 20, 1914, to April 20, 1915.

One set was kept at room temperature and the other set was placed outside, subject to atmospheric temperatures.

Six jars (3 for each set) of glazed earthenware, holding about 1850–1900 gm. of soil, were used. These were filled with soil prepared as follows: nos. 1 and 2, Miami silt loam containing 23 per cent moisture; nos. 3 and 4, Miami silt loam containing 23 per cent moisture and 1 per cent dextrose; and nos. 5 and 6, Miami silt loam containing 28 per cent moisture.

The soil was thoroughly mixed and the moisture raised to the proper amount, care being taken to secure a uniform moisture content throughout the sample. Muslin was tied over the tops of the jars, which allowed free access of air but which excluded dust. Jars 2, 4, and 6 then were placed outside of a window, and protected from rain and snow by a large bell jar which allowed free circulation of the air. Jars 1, 3, and 5 were kept in a room at an average temperature of about 25° C. The jars were left untouched for a period of 18 days (February 16–March 6). The average daily temperatures for this period which affected the outside jars are shown in graph 2.

At the end of the period an average 20 gm. sample was taken from each of the jars and treated in the same way as the field soil samples previously described. Plates poured with Heyden Nährstoff agar and counted after 8 days gave the results indicated in table II.

TABLE II

No. of jar	Treatment	Percentage of moisture content of soil	Average number of colonies per plate*	Bacteria per gm. dry soil
1	23 per cent H_2O	8.42	51	5,471,994
2	23 per cent H_2O (frozen)	15.00	48	5,548,800
3	23 per cent H_2O 1 per cent dextrose	9.99	261	28,491,543
4	23 per cent H_2O 1 per cent dextrose (frozen)	15.80	155	18,089,275
5	28 per cent H_2O	9.31	61	6,609,167
6	28 per cent H_2O (frozen)	17.48	51	6,073,131

*Fourth dilution.

It should be noted that in the cases where the soil was kept in the room, the evaporation was much more rapid than in the soils kept outside, so that while the original moisture content was the same, when the counts were made the amount of moisture present in the jars kept outside was nearly double that in the jars kept in the room.

The results recorded in table II show clearly the retardation in growth which was caused by cold and frost. In the case of jars nos. 1 and 2, the number of bacteria per gm. of soil is almost the same in the two samples, yet sample no. 2 has only slightly less than twice as much moisture as sample no. 1. Jars nos. 3 and 4 show the retardation even better. Here 1 per cent of dextrose was added so as to produce a rapid growth. No. 3 with a much lower moisture content than no. 4 shows a much greater increase in growth. This is the reverse of what might be expected to happen under ordinary conditions, and must be ascribed to the action of frost, since normally the bacterial content should rise with an increase in moisture. Jars nos. 5 and 6 gave results similar to jars nos. 1 and 2, the number of bacteria being slightly higher because of the higher moisture content.

In general these figures show that even a much higher moisture content was not sufficient to counteract the retarding in growth due to cold.

Types of bacteria in frozen soil

No detailed study of the varieties of bacteria in the soil was made, since this would have required much more time than was at the writer's disposal. Nevertheless, general differences in the kinds of colonies present in successive counts were noticed.

Usually the plates contained a varying number of raised colonies of yellow, red, and fluffy white actinomycetes, a few spreaders of the *B. mycoides* type, some red or yellow chromogens, numerous small, slow-growing, transparent colonies, and many white or cream colored colonies without distinctive marks, some of them raised and others flat. Occasionally the plates showed a few opalescent colonies.

So far as could be noticed from the examination of colony growths, there was no distinct difference between the fall, winter, and spring floras. It was noticeable, however, that the relative proportions of the different types sometimes changed markedly in successive counts. Thus it was found that after heavy frosts the small transparent colonies made up sometimes one-half or more of the total number of colonies on the plates. In subsequent counts they would gradually decrease in number.

The actinomycetes and the spreaders did not seem to be affected by the frost, being present on nearly every plate counted during the winter. The chromogens were more irregular in their occurrence and apparently were somewhat less abundant during very cold periods.

The writer wishes to express his indebtedness to Professor E. B. FRED, University of Wisconsin, for helpful suggestions and criticisms.

UNIVERSITY OF WISCONSIN
MADISON, WIS.